Description of the Invention

The radiotherapeutic agent may be any of a variety of nuclides, depending on the energy, penetration or half-life that is desired. Appropriate choice of such criteria is within the skill of those knowledgeable in the art. It is preferably a ferrite, e.g. a β-emitting ferrite, and preferably comprises a chelate.

Suitable tissue glues are also known. This component may be, for example, based on a clottable protein such as fibrinogen. For example, a glue such as Tisseel (available from Immuno Danmark A/S of Copenhagen) may be used. Tissue glues, including fibrin and cyanoacrylates, are also described by, for example, Kottai et al, Ann. Otol. Rhinol. Laryngol. (1983 Jan-Feb) 92(1 Pt 1):29-32; Ronis et al, Laryngoscope (1984 Feb) 94(2 Pt 1):210-3; Barnstable, Nature (1986 Jun 19-25) 321 (6072):731-2; Toriumi et al, Otolaryngol. Clin. North Am. (1994 Feb) 27(1):203-9; and Schlag et al, in Fibrin Sealant in Operative Medicine Vo. 1, G. Schlag. 11. Redl (eds), Springer-Verlag Berlin-Heidelberg (1986) 27-38.

The term "tissue glue" is used herein in its broad sense, i.e. as any tissue-compatible matrix within which the active component is retained, suitable for topical or other application to the locus of treatment. It may therefore be a gel-like substance comprising pores within which the agent is held. It will often be proteinaceous, and usually it will be biodegradable.

According to one aspect of this invention, it has been appreciated that larger particles can readily be immobilised in a variety of gels such as the tissue glue. Further, these particles are made in such a way that they can be fully and stably dissolved in water or in e.g. the calcium fluid component of the tissue glue, yet these particles are small enough to be sterilised by filtration methods along with the solution in which they are dissolved. The particles can be manufactured in such a way as to incorporate a wide variety of different metal

cations, including their radioactive isotopes (to include emitters of β-particles, α-particles, γ-rays, or X-rays from K-capture decay) in the ferrite crystal matrix at their core. The nuclides are very stably incorporated into the ceramic type core of 10-200 nanometers in diameter and the core is coated in any one of a variety of macromolecules such as dextran.

These particles can be made radioactive in several different ways. It is only necessary to dissolve radioactive isotopes of certain metals as soluble metal chlorides in the initial metal chloride solution prior to the ferrite precipitation step disclosed in WO-A-9305815 and US Application Serial No. 08/211,041, filed March 16, 1994, the content of which is incorporated by reference.

The isotope palladium-103 ($t^{\frac{1}{2}}$ = 17 days, EC) is extremely useful for brachytherapy with permanent implants because of its useful half-life which allows treatment of a cancer as various cells in the tumour pass into the radiosensitive cell division process over the course of days and weeks. The X-rays produced by its electron capture decay have low energy (half-value thickness in water of 1.6 centimetres) and hence it is possible to have exceedingly precise control over the area treated, as for example in the post-excision area where invisibly small amounts of tumour may have invaded surrounding normal tissue to a depth of a few millimetres. It is also the case, as is disclosed in WO-A-9211846 and US Application Serial No. 08/087,781, filed July 7, 1993 (the content of which is incorporated by reference) that palladium is very readily incorporated into spinel ferrites in stable fashion.

Two readily available methods exist for the production of ^{103}Pd. The first is by the use of low energy neutrons from a production reactor to irradiate the ^{102}Pd stable isotope. It should be noted that this method has the disadvantage that ^{102}Pd is only one of five different stable isotopes of palladium (see Table 1) and makes up only 1% of

naturally-occurring palladium. For reasons discussed below, it is necessary to extract the 102 isotope prior to irradiation because of the undesirable qualities of the other resulting radioisotopes if the full mixture of stable palladium isotopes is irradiated (see Table 2). The second method for production of ^{103}Pd is to use a high energy cyclotron capable of causing reactions with protons (conventional cyclotrons use deuterons and alpha particles which have greater mass/charge). Such an instrument can be used to irradiate rhodium which occurs naturally as only a single isotope, ^{103}Rh, so that all conversions are to ^{103}Pd which is then separated chemically from the rhodium in carrier free form.

Another medically useful nuclide for delivery with ferrite or other particles is yttrium-90 ($t^{½}$=64 hours, β-, 2.283 MeV). This is readily produced from low energy neutron irradiation of ^{89}Y since 100% of natural yttrium occurs in this form. The pure beta emission and half-life are suitable for such tasks as radiation synovectomy and treatment of other very thin layers of disordered tissue. The provision of ^{90}Y in an appropriate liquid carrier has been a problem for many years since, for intra-articular use, small molecule carriers and colloids tend to leak out of the joint, while large particles do not distribute properly because they do not remain in solution. It has been suggested previously in WO-A-9211846 (see above) that various lanthanides can be incorporated into garnet ferrite-type crystals. For the inclusion of yttrium, a second period Group IIIB element, it has been appreciated that the stability and efficiency of incorporation can be improved by forcing the yttrium into a modified spinel crystal.

The prototype mineral spinel ($MgAl_2O_4$) is based on a close-packed, face-centred, cubic crystal of oxygen atoms with metal ions placed at interstitial spaces in the crystal. There are A spaces which accommodate ions of 0.3 to 0.6 angstroms (8 sites/subunit) and B spaces which

radioactive material becomes subject to leakage out of the joint. To minimize this problem, patients are now required to remain motionless in bed in hospital for several days.

The incorporation of the radioactive yttrium into ferrites makes possible several methods of limiting the leakage and subsequent spread of radioactivity. Firstly, the knee can be wrapped in a magnetic coil which will prevent any leaked radioactive ferrites from spreading out of the area. More simply, however, tissue glues can be used to help seal the joint capsule. Because the radioactivity is incorporated in a substance which does not diffuse through tissue glue, the application of tissue glue to the injection site will prevent leakage. The half-life of the ^{90}Y is sufficiently short that most of the radioactivity will have decayed before the tissue glue will have broken down. In this aspect, the invention provides a system which inhibits unwanted spread of synovectomy agent.

In a further modification, the ^{90}Y-ferrites may be fully incorporated into the tissue glue at the time of injection into the joint via a double-barrelled, double-needle syringe. The, say, fibrin glue is allowed to set within the involved joint, but its injection is accompanied by agents which help promote fibrinolysis, such as tissue plasminogen activator, or it is applied in a specially modified tissue glue preparation which is compounded to hold a lower concentration of fibrinogen then is usually used. In this fashion it can be assured that the ferrite-containing gel will dissolve over 2-4 days during which time much of the activity of the ^{90}Y will be retained. During the breakdown process, macrophages are known to be actively attracted into the matrix and will ingest radioactive particles as well. This will thus serve as a means of both reducing leakage from the joint and effectively loading macrophages with radioactive ferrites so that when they subsequently distribute to the synovium they will be highly active.

this treatment since it would be converted into a very long half-life, low energy beta-emitter (^{107}Pd, 6.5 x 10^6 y, β-, .033 MeV, decaying to stable ^{107}Ag). However, for the preparation of permanent implants, this would be a desirable effect, and natural, mixed isotope palladium could be used as a starting material. The small amount of ^{103}Pd produced would decay to negligible amounts after ten half-lives (170 days), after which the material could be treated as an effectively permanent source of low energy beta particles. In yet another aspect, because of the extremely low energy of these beta particles, the use of a plastic or silastic capsule or a titanium capsule around a seed cast to include such ferrites, or a thin, e.g. 1 mm, layer of ferrite-free tissue glue would provide nearly complete shielding of the beta emissions. In this way, the natural palladium ferrite could still be used as a permanent implant emitting effectively only the desirable X-rays from electron capture decay of ^{103}Pd. The ^{109}Pd (13h,β-,1 MeV) produced from natural ^{108}Pd would still make it necessary to hold the particles for at least ten of these half-lives (130 hours) to reduce this beta output if it was desired to use only EC X-rays. However, in many therapeutic situations, inclusion of a 1 MeV beta particle would be useful along with the EC X-rays.

Fast neutron bombardment of ^{56}Fe-enriched ferrites is another feasible means of producing useful medical isotopes via the (n,p) reaction. The various nuclides produced form the dextran-coated ferrite carrier are all very short-lived (see Table 3).

Use of a high energy cyclotron to bombard ^{56}Fe-enriched ferrites with protons results in ^{56}Co which is a relatively long half-life (77 days) positron-emitter. In both these cases, the advantage is in the ability to incorporate the radioactivity after the chemical preparation, clean up, and concentration steps have been completed. This avoids exposure to those involved in preparing the material and is particularly helpful in preparing positron-emitting

ferrites since it is extremely difficult and awkward to shield production workers from positron annihilation photons due to their high energy.

In yet another aspect, the bombardment of ^{56}Fe-enriched dextran-coated ferrites with alpha particles would produce ^{58}Ni (α,2n) which is stable but would have the advantageous effect of converting oxygen atoms in the ferrite crystal as well as in the dextran into the short half-life (110 min) positron-emitter ^{18}F (α,pn) which has a wide variety of medical uses; however, the side-reaction of (α,pn) production of ^{58}Co (70d,β+) limits the value of this production method. Treatment of dissolved dextran by alpha bombardment prior to the precipitation reaction would cause the inclusion of ^{18}F in both the dextran coat and in the interior of the crystal.

A completely different way of adding radioactivity after manufacture of the ferrites or of any other useful particle of the desired size range is to conjugate chelating groups such as EDTA, NTA or DTPA to the dextran coat of the ferrite or surface group of any other particle. In this fashion, all preparation steps are done with non-radioactive materials. Finally, when the material is to be shipped for use, or even at the site of use, the chelate-conjugated particles are exposed to dissolved radioactive metals or other chelatable elements. The elements need only have higher binding affinity to the chelate than e.g. sodium used to fill the chelate prior to radioactive uptake.

After exposure of an excess of the particles to the radioactive material, it is optionally possible to use centrifugal ultrafilters or simply passage through Sephadex columns to separate the hot particle fraction from any unbound radioactive elements.

It should also be noted that the considerations discussed above concerning palladium isotopes also apply in this alternative situation in which a reactor is used to irradiate natural mixed palladium before preparation of the

solution. Other macromolecules such as proteins, fibrin, collagen, starch, polylysine, or derivatized dextrans may also be used for the coating. Alternatively, a trivalent lanthanide or Group IIIB chloride may be substituted for 10-50% of the $FeCl_3$. A variety of unstable isotopes of various transition, lanthanide and actinide elements can be introduced at this step as metal chlorides dissolved in water or in an acidic solution of e.g. 0.1M HCl. The amount of trivalent iron may be reduced where trivalent cations are added.

Dissolve 100 mg $FeCl_2.4H_20$ in the Fe_3/dextran solution then place the mixture in a 60°C water bath for two minutes. Some divalent cations may be added in place of Fe at this step. Some metal chlorides such as, e.g. palladium chloride will require an extended period of time up to e.g. one to two days in concentrated HCl in order to become fully dissolved prior to mixing with the other constituents. When trivalent lanthanides are used, the crystal structure may be modified by the use of copper or magnesium as the divalent cation instead of the $FeCl_2$, or other stable combination of cations such as $FeCl_2$ with $ZnCl_2$ may be used to help accommodate Group IIIb elements such as the yttrium^{90} isotope. Further, the reaction mixture may be allowed to stand at room temperature or be cooled as low as 0-4°C prior to proceeding depending upon the desired size distribution of the resultant particles where cooler reaction temperatures tend to produce smaller particles.

Gradually add 6 ml of hot 7.5% NH_3 solution (60°C). The NH_3 solution may also be added at lower temperatures, e.g. 0-4°C or room temperature. Equivalence of temperature and mixing to assure uniform concentration as well as more gradual and dispersive addition of the NH_3 solution will help reduce the range of particle sizes. The product is left to stand in the 60°C water bath for fifteen minutes for Fe/Fe particles, but the incubation may be extended up to four hours when lanthanides or Group IIIB elements in substantial amount are used in place of $FeCl_3$.

The reaction product (dextran-coated ferrites) is spun at 1,000 g for 10 minutes and any precipitate is discarded. This process is repeated to complete three spins and the supernatant then applied to PD-10 columns equilibrated with 0.1M Na Acetate buffer pH 6.8 with 5mM EDTA.

The black eluted fraction is diluted 1:3 with EDTA/Acetate buffer then concentrated to 1/10 of the initial volume with Amicon Centriprep-100 ultrafilters. The retentate is then diluted 1:10 with EDTA/Acetate buffer then concentrated to a volume of 1.5 ml with the C-100 ultrafilters.

At this point the preparation may be stored or further concentrated. If it is to be used with a tissue glue, it can be mixed with one component of the fibrinogen, fibrin, collagen, acrylic, gelatin, resorcinol or other type of tissue glue, matrix, or polymer precursor prior to application and mixing of the glue to set on the tissue surface.

When it is desired to attach an additional ligand such as for instance an antibody, lectin, or other agent which promotes adherence to the glue matrix, the following steps as well as other methods well known to those expert in the art of conjugation to dextran may be used.

Add 0.30 ml of 20 mM $NaIO_4$ to the dextran ferrite solution (approx. 1.5 ml) while stirring then gently tumble or shake for 60 minutes at room temperature in the dark.

At the end of the 60 minute periodate incubation, the reaction is terminated by applying the reaction mixture to the PD-10 columns equilibrated with 20mM borate buffer (pH 8.5).

An active site blocking solution is prepared using 100 mM $MnCl_2/CaCl_2$ for WGA binding reactions and appropriate blocking agent for any other targeting protein to be conjugated to the particle.

Dissolve 10 mg of the protein (e.g. cell targeting protein or antibody) in 500 μl of 20mM NaBorate buffer pH 8.5 at room temperature. The protein solution can be

diluted to 12 ml with borate buffer, then concentrated with Centriprep-10 concentrators to remove DTT, glycerol, NaN_3 and other undesirable storage additives.

Add 10 µl of the blocking solution to the protein/borate solution then mix 2.0 ml of oxidized magnetite dextran with 500 µl of the protein/borate solution. Pipette 20 µl blocking solution into the 2.5 ml protein-dextran-magnetite mixture and mix well, then incubate for 6-18 hours at room temperature in a gentle tumbling or shaking device.

After the incubation add 100 l of 0.5M glycine to the reaction mixture and incubate an additional 2 hours. Then add 250 µl of 0.25M $NaBH_4$ to the magnetite-dextran-protein solution and allow to stand for 60 minutes, shaking periodically to release H_2 gas. At the end of the incubation, pass the reaction mixture through PD-10 columns equilibrated with 20mM HEPES buffer, pH 7.4. Dilute the eluant 1:5 with HEPES buffer then concentrate with Centriprep-100 ultrafilters.

An affinity purification step is optional and detail is given for use with a WGA(lectin) targeting protein by example. Apply final retentate to affinity columns (20 mM HEPES), wash with HEPES then carry out specific elution with 1M NAcGlu in HEPES buffer pH 7.4. Pass the specific eluant through PD-10 columns equilibrated with HEPES to remove NAcGlu, Mn and Ca.

The desalted output is then diluted to a volume of 24 ml with HEPES buffer and concentrated with Centriprep-100 concentrators. The final retentate form is sterilized by spinning at 500 g for one hour in 0.22 µm centrifugal microfilters.

Brief Description of the Drawings

Figure 1 shows a balloon-tipped catheter system, for temporary introduction of liquid brachytherapy agent, including a catheter 1, collapsed balloon tip 2, stopcock 3, an adapter 4, radiotherapeutic liquid 5 and an immiscible 'chaser' fluid 6. Fig. 5A shows the parts of

the system; the four stages of its use are: B) the assembled system with air evacuated from the catheter and balloon, both being collapsed, the stopcock valve maintaining a seal, C) a column of radiotherapeutic fluid being pumped into the catheter, D) the fluid reaching and starting to fill the balloon tip, E) the balloon tip fully filled with radiotherapeutic liquid and non-radioactive immiscible chaser fluid filling the catheter up to the balloon entry point. Optionally, a second tube may be used to fill an internal balloon, or the first tube used to send down a leading column of fluid to dilate an internal balloon with the purpose of creating a thin shell of radioactive fluid against the inner surface of the outer balloon.

Figure 2 shows a lead-shielded double-syringe device for applying a thin sheet of radiotherapeutic tissue glue. The syringes include barrels, one indicated at 7, a wheel 8 placed between the terminal portions of the dispenser plates to maintain a uniform distance from the tissue surface, an optional wheel 9 with penetrating feet, an optional wheel 10 with a thin silastic outer rim, and two dispenser plates 11 seen in lateral view, one behind the other. Fig. 2F is a lateral view of the system without the spacing wheel; Fig. 2G is a lateral view of the system with one of the optional wheels in place, and Fig. 2H is an anterior view of the assembled double-syringe system showing the appearance of one of the various widths and shapes of disposable dispenser plates which may be affixed to the syringe system.

Figure 3 shows a device for distributing therapeutic adhesive within a tumor mass, including a sheath 12, a plunger shaft 13, a plunger/manifold cover 14, needles 15 (at 15b, viewed from above with four double-barrelled needles extended), double-barrelled syringe 16, connecting tubing 17 for carrying glue components to a manifold 18 dividing flow of glue components into four sets and then pairing up the sets of double-barrelled needles, one pair

of needles shown at 19 emerging from manifold cover to enter the plunger shaft, and, shown at 20, a fully-extended set of double-barrelled needles spreading out from sheath tip to penetrate tumor in various directions. Fig. 3J shows the plunger lifted to withdraw needles back into the sheath. Fig. 3K shows the plunger advanced to force the four double-barrelled needle sets out into the tumor in various directions, and Fig. 3L shows the syringe manifold system which allows distribution of the two component glue into the double-barrelled needles after they are in place and while they are being withdrawn.

11. A method for the radiotherapy of a tumor, which comprises applying to the tumor an effective amount of a composition as defined in claim 1.

12. The method of claim 11, wherein the radiotherapy of a tumor comprises brachytherapy.

13. A composition comprising an antibody, a particulate radionuclide and a fibrinogen tissue glue.

14. The composition according to claim 13, wherein the particulate radionuclide is a β-emitting ferrite.

15. The composition according to claim 13, wherein the particulate radionuclide is coupled to the antibody.

16. The composition according to claim 15, wherein the antibody is a nerve adhesion molecule.

17. A method for making a radiotherapeutic composition comprising an antibody, a particulate radionuclide and a fibrinogen tissue glue which comprises:

(a) preparing a particulate radionuclide; and

(b) mixing the particulate radionuclide with the fibrinogen tissue glue and the antibody.

18. A method of using a radiotherapeutic composition comprising an antibody, a particulate radionuclide and a fibrinogen tissue glue which comprises applying the composition directly to tumor tissue.

19. A method of radiation synovectomy which comprises administering an effective amount of a composition of claim 1 to a patient to be treated.

20. A method of radiotherapy in the treatment of arterio-venous malformations in a blood vessel which comprises applying to the blood vessel a composition as defined in claim 1.